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Utility of MTT assay in three-dimensional cultured human skin model as an alternative for Draize skin irritation test: Approach using diffusion law of irritant in skin and toxicokinetics-toxicodynamics correlation

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Purpose. A cytotoxicity assay using a three-dimensional cultured human skin model, Living Skin Equivalent-high (LSE-high) was evaluated as an alternative to the Draize skin irritation tests using animals. A relation between the cytotoxicity and calculated concentration of an irritant in skin was also evaluated.
Methods. Colorimetric thiazoyl blue (MTT) conversion assay and a surfactant, cetylpyridinium chloride (CPC), were selected as a cytotoxicity assay and a model irritant. The fraction of dead cell number in the MTT assay or the Draize irritation score (*in vivo* and *in vitro* irritation data, respectively) was treated as a function of CPC concentration in the viable skin of LSE-high and guinea pig. Separately, *in vitro* permeations of CPC through the LSE-high or excised guinea pig skin were determined to calculate the average concentration of CPC in the viable skin using the Fickian diffusion theory. The obtained relations between the irritation score and CPC concentration were evaluated by the Emax model (Hill equation).
Results. CPC concentration showing 50% irritation (IC_{50}) was similar for the MTT assay (18.9%) and Draize test (12.3%), and a good relationship ($r = 0.981$) was observed between the fraction of dead cell number and the Draize score. In contrast, IC_{50}, 1.32%, for the MTT assay in LSE-high was much lower than that using guinea pig skin. We then corrected the results for the MTT assay using a

ratio of IC_{50} in guinea pig skin against LSE-high, resulting in a good relation between both MTT results in guinea pig skin and LSE-high.

Conclusion. The present results suggest that the MTT assay using LSE-high may be utilized as an alternative for the Draize test in animals for evaluating skin irritation.